

# No consistent evidence for association between mtDNA variants and Alzheimer disease

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Supplemental Data



## ABSTRACT

**Objective:** Although several studies have described an association between Alzheimer disease (AD) and genetic variation of mitochondrial DNA (mtDNA), each has implicated different mtDNA variants, so the role of mtDNA in the etiology of AD remains uncertain.

**Methods:** We tested 138 mtDNA variants for association with AD in a powerful sample of 4,133 AD case patients and 1,602 matched controls from 3 Caucasian populations. Of the total population, 3,250 case patients and 1,221 elderly controls met the quality control criteria and were included in the analysis.

**Results:** In the largest study to date, we failed to replicate the published findings. Meta-analysis of the available data showed no evidence of an association with AD.

**Conclusion:** The current evidence linking common mtDNA variations with AD is not compelling. *Neurology*® 2012;78:1038-1042

## GLOSSARY

**AD** = Alzheimer disease; **DSM-IV** = *Diagnostic and Statistical Manual of Mental Disorders, 4th edition*; **GERAD1** = Genetic and Environmental Risk for Alzheimer's Disease Consortium 1; **LHON** = Leber hereditary optic neuropathy; **MMSE** = Mini-Mental State Examination; **MRC** = Medical Research Council; **mtDNA** = mitochondrial DNA; **SNP** = single nucleotide polymorphism.

Both genetic and environmental factors contribute to the risk of developing Alzheimer disease (AD), with heritability estimates of up to 79%.<sup>1</sup> Variants in 3 genes (*APP*, *PS1*, and *PS2*) cause rare Mendelian forms of the disease, and 10 loci increase susceptibility for the more common late-onset form.<sup>2</sup> Although known genetic variants account for 32% of the genetic variation in AD, most of the genetic variance associated has yet to be attributed to specific loci.

Progressive mitochondrial dysfunction has been reported in the postmortem AD brains<sup>3</sup> and non-neural tissues,<sup>4</sup> implicating a systemic defect of oxidative phosphorylation. Thirteen essential respiratory chain proteins are synthesized from maternally inherited mitochondrial DNA (mtDNA). Several studies have reported the association of different mtDNA haplogroups or specific mtDNA single nucleotide polymorphisms (SNPs) with AD, with both concordant and conflicting results (table 1). Many of these studies were small and had limited power, but 2 of the larger studies reached different conclusions. In 170 AD case patients and 188 controls, mt.9698T, mt.11467G, mt.12308G, mt.12372A, and mt.16270T were associated with AD.<sup>5</sup> These SNPs are found almost exclusively with haplogroup UK. However, in 936 AD case patients and 776 controls, mt.4336C and mt.15883T were associated with AD<sup>6</sup> and fall within haplogroup H. mt.4336C defines

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**Study funding:** Supported by the Wellcome Trust, Medical Research Council (UK), Alzheimer's Research UK, Welsh Assembly Government, and Newcastle NIHR Biomedical Research Centre.

**Disclosure:** Author disclosures are provided at the end of the article.

**Table 1** Published studies of mitochondrial DNA in Alzheimer's disease<sup>a</sup>

Author and year	Journal	Patients	Controls	Variant	Haplogroup	p Value	OR	Gender
Lakatos et al., 2010 <sup>5</sup>	Neurobiol Aging	170	188	mt.11467G	UK	0.003	2.22	
				mt.12308G	UK	0.006	2.03	
				mt.12372A	UK	0.006	1.99	
				mt.9698T	u8	0.021	2.26	
				mt.16270T		0.048	2.52	
Santoro et al., 2010 <sup>6</sup>	PLoS One	936	776	mt.4336C and mt.15883T	H5	0.001 <sup>b</sup>	1.85	
					H5	0.033 <sup>b</sup>	2.19	F
Kruger et al., 2010 <sup>18</sup>	Mol Neurodegener	128	99		IWX	0.03	2.69	
Maruszak et al., 2009 <sup>19</sup>	Neurobiol Aging	222	252	mt.7028C and mt.4580A	HV	0.03 <sup>b</sup>	1.59	
Mancuso et al., 2007 <sup>20</sup>	Neurol Sci	209	191			No association identified		
Elson et al., 2006 <sup>21</sup>	Hum Genet	260	243			No association identified		
van der Walt et al., 2004 <sup>22</sup>	Neurosci Lett	989	328	mt.10398A and mt.12308G	U	0.04 <sup>b</sup>	2.30	M
				mt.12308G	U	0.02 <sup>b</sup>	0.56	F
				mt.7028T	Non-H	0.05 <sup>b</sup>	0.66	F
Carrieri et al., 2001 <sup>23</sup>	Hum Genet	213	989	mt.7028C	H		2.89 <sup>c</sup>	
				mt.10550G	K		9.69 <sup>c</sup>	
				mt.4917G	T		2.66 <sup>c</sup>	
Chagnon et al., 1999 <sup>24</sup>	Am J Hum Genet	69	83	mt.709A	T	0.01		
				mt.15928A	T	0.04		

Abbreviation: OR = odds ratio of association.

<sup>a</sup> The table shows the principal author, year of publication, journal, number of samples used in the study (both patients and controls), the variants associated with Alzheimer disease and the corresponding haplogroup determined from mitomap.org, probability of association (*p* by  $\chi^2$  test unless stated), and OR and whether the effect was gender-specific (M or F).

<sup>b</sup> By logistic regression (including; gender, age, and APOE4 status).

<sup>c</sup> APOE4 patients only.

subhaplogroup H5a, which can then be further subtyped into subhaplogroup H5a1, based on mt.15833T.<sup>6</sup> In an attempt to resolve this issue, we tested mtDNA variation for association with AD in a large cohort of AD case patients and age-matched controls from 3 Caucasian populations.

**METHODS** We studied 138 mitochondrial variants present on the Illumina 610-Quad chip genotyped in 4,133 AD case patients and 1,602 elderly, ethnically matched controls from the United Kingdom, United States, and Germany as part of the Genetic and Environmental Risk for Alzheimer's Disease Consortium 1 (GERAD1) study.

The GERAD1 sample has been extensively described elsewhere.<sup>1</sup> These samples were recruited by the Medical Research Council (MRC) Genetic Resource for AD (Cardiff University; Institute of Psychiatry, London; Cambridge University; and Trinity College Dublin); the Alzheimer's Research UK Collaboration (University of Nottingham; University of Manchester; University of Southampton; University of Bristol; Queen's University Belfast; and the Oxford Project to Investigate Memory and Ageing, Oxford University), MRC PRION Unit, University College London; London and the South East Region Alzheimer

Disease project, University College London; Competence Network of Dementia and Department of Psychiatry, University of Bonn, Bonn, Germany; Washington University, St. Louis, Missouri; and the National Institute of Mental Health AD Genetics Initiative. AD case patients met the criteria for either probable (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association<sup>7</sup> and *DSM-IV*) or definite (Consortium to Establish a Registry for Alzheimer's Disease<sup>8</sup>) AD. Controls were screened for dementia using the Mini-Mental State Examination or Alzheimer's Disease Assessment Scale–Cognition, were determined to be free from dementia at neuropathologic examination, or had a Braak score of  $\leq 2.5$  (table e-1 on the *Neurology*<sup>®</sup> Web site at [www.neurology.org](http://www.neurology.org)).

All DNA samples were genotyped at the Sanger Institute (Cambridge, UK) on the Illumina 610-Quad chip. Included in the array were the variants used to define haplogroup H5 and its subgroups, H5a and H5a1 (mt.456C>T, mt.4336T>C, and mt.15833C>T, respectively) and 4 variants found on haplogroup UK (mt.11467A>G, mt.12308A>G, mt.12372G>A, and mt.9698C>T), which were previously associated with AD.<sup>5,6</sup> Stringent quality control filters were applied to remove poorly performing samples using tools implemented in PLINK v1.05 (<http://pngu.mgh.harvard.edu/~purcell/plink>).<sup>19</sup> We excluded individuals with

missing genotype rates  $>0.01$ , those with inconsistencies between reported gender and genotype-determined gender or ambiguous genotype-determined gender, or those who appeared to be of non-European ancestry. We also examined genetic relatedness and only retained one of each pair of individuals with an identity-by-descent estimate  $\geq 0.125$  (the level expected for first cousins). After quality control, 3,250 case patients and 1,221 elderly controls remained. We studied all 138 mitochondrial SNPs, including low-frequency variants (minor allele frequency  $>0.01\%$ ). Variant frequencies were compared in case patients and controls: 1) on an individual SNP-by-SNP basis using Pearson's test ( $p$ ) and 2) across the entire data set by permuting the disease status ( $p^*$ ), an approach that partially accounts for the phylogenetic structure of the data. All statistical analysis was carried out in PLINK (v2.050) using a single allele-based model. Published data reporting the same mtDNA SNPs were compiled into a single pooled analysis using the same statistical approach. Power calculations were performed using Genetic Power Calculator.<sup>10</sup>

**Standard protocol approvals, registrations, and patient consents.** This study received national ethical approval. Written informed consent for the research was obtained for all patients who were participating in the study.

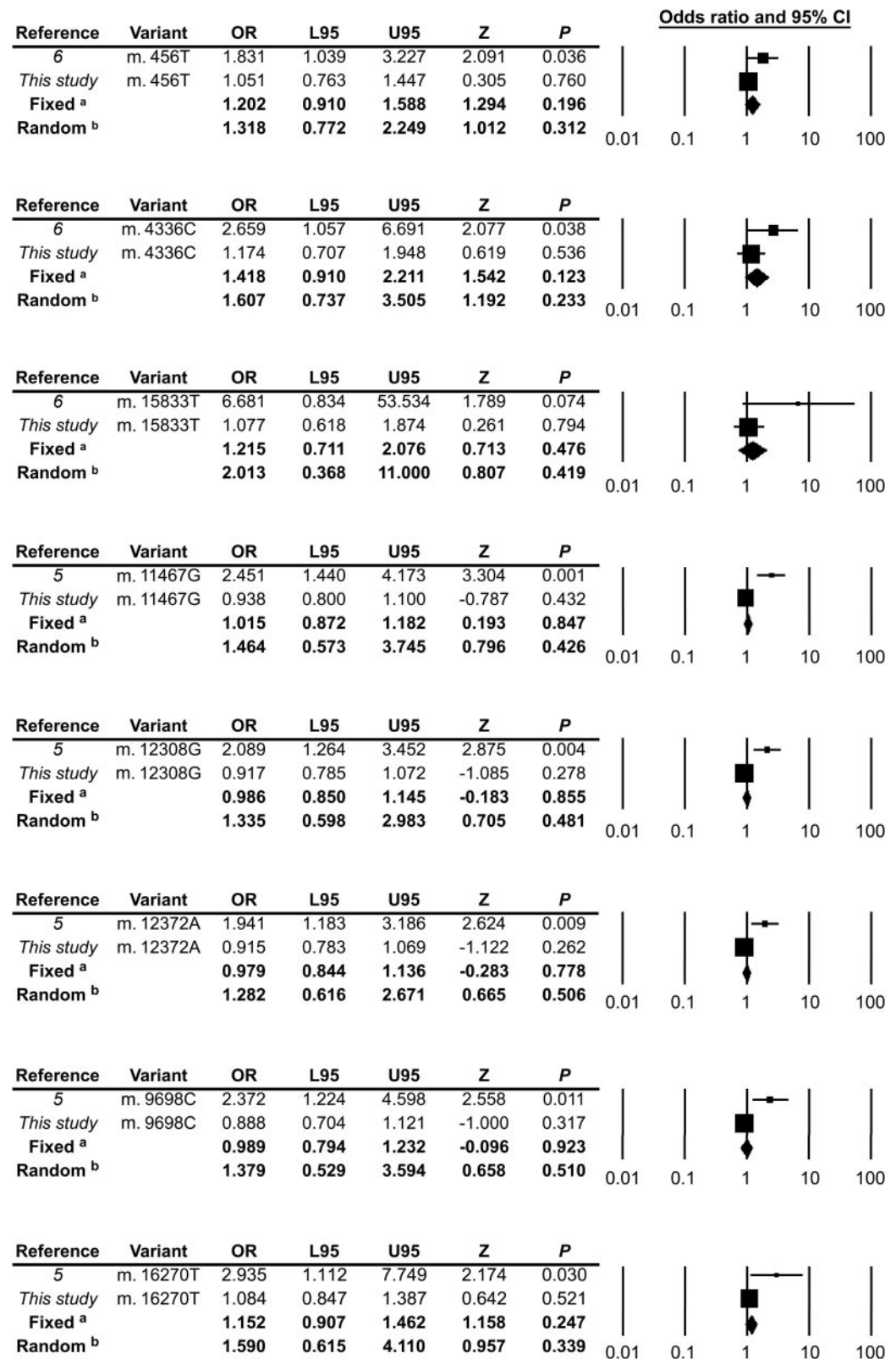
**RESULTS** We observed some evidence for association between individual mtDNA SNPs previously implicated and AD within subsets of samples, dependent on geographical location. However, no single SNP was consistently associated with AD across all 3 cohorts, and the  $p$  values did not withstand a Bonferroni correction to account for multiple statistical testing. Permutation analysis of the entire dataset also showed no significant association between any one SNP and AD, either within each cohort in isolation or when all 3,250 AD case patients and 1,221 controls were pooled. There was no evidence of gender-specific association with any SNP nor any evidence of an interaction with *APOE*. Power calculations showed that we had  $>80\%$  power to detect the previously reported associations<sup>5,6</sup> (assuming 2-tailed significance and  $\alpha = 5\%$ ) with mt.4336C (power = 83.0%), mt.9698T (100%), mt.11467G (100.0%), mt.12308G (100.0%), mt.12372A (100.0%), mt.15833T (94.7%), and mt.16270T (100.0%), even though the control group was smaller than the disease group. A meta-analysis of the current data and previously published studies showed no evidence of association between these 7 variants and AD (figure).

**DISCUSSION** We report the largest study of mtDNA variation in AD to date. In addition to the major European haplogroups, our data include variants that define subhaplogroup H5 and its further subdivisions H5a and H5a1 (mt.4336T $>$ C and mt.15833C $>$ T, respectively), along with 4 vari-

ants found on haplogroup UK (m.11467A $>$ G, m.12308A $>$ G, m.12372G $>$ A, and m.9698C $>$ T). Our findings fail to replicate previous studies reporting associations with either single genetic variants or specific mtDNA haplogroups.

How can we explain the previous findings? The strict maternal inheritance of mammalian mtDNA and the associated lack of intermolecular recombination renders mtDNA genetic association studies particularly vulnerable to a population stratification effect.<sup>11</sup> This increases the chance of detecting a false-positive disease association.<sup>12</sup> In addition, given that the size of any genetic effect is likely to be small, a reliable association study requires a very large sample size to deliver a consistent result.<sup>13</sup>

Although our findings show that the evidence linking inherited mtDNA variants to AD is not compelling, the relative contribution of specific mtDNA variants could vary in different ethnic groups, possibly through an interaction with environmental factors and different nuclear genes.<sup>14</sup> In practice, this means that the specific mtDNA variants that fail to show an association with disease in this study could be associated with disease in a different ethnic population. Geographic variation in allelic association could also arise through homoplasmy. Homoplasmy is the recurrence of mutations on different branches of the mtDNA phylogeny in different parts of the world. Homoplasmy accounts for up to 20% of mtDNA variation and often involves nonsynonymous substitutions.<sup>15</sup> This raises the possibility that haplogroup markers tag different homoplastic functional variants in different populations. If the homoplasies are having a functional effect, this would lead to different haplogroup associations in different studies across the globe. Finally, it is possible that geographic differences in the fine detail of the subhaplogroup structure of mtDNA could account for inconsistencies between studies. This situation has been described for the primary mitochondrial disorder, Leber hereditary optic neuropathy (LHON). LHON is a maternally inherited form of blindness primarily due to 1 of 3 mutations of mtDNA: mt.11778G $>$ A, mt.14484T $>$ C, or mt.3460G $>$ A. The clinical penetrance of LHON is influenced by common polymorphic variants of mtDNA.<sup>16</sup> Specific subbranches of haplogroup J are associated with either an increased or decreased risk of visual failure in different populations, largely due to specific differences in the cytochrome B protein sequence.<sup>17</sup> A similar situation could exist for AD, but resolution of the issue will only be possible through high-resolution genotyping in very large cohorts of patients and carefully matched controls, ideally at the whole mtDNA genome level.



Results for the fixed<sup>a</sup> and random<sup>b</sup> effects models are shown. The size of the central box on the figure corresponds to the relative study size in each case. The diamond shows the results of the meta-analysis. Current = data from the study reported here; L95 = lower 95% confidence interval; OR = odds ratio; U95 = upper 95% confidence interval; Z = z score.

## AUTHOR CONTRIBUTIONS

Prof. Williams directed this study. Dr. Hudson, Prof. Chinnery, Prof. Williams, and Dr. Sims took primary responsibility for drafting the manuscript assisted by Prof. O'Donovan and Prof. Owen. The GERAD Consortium, Prof. Williams, Dr. Sims, Dr. Harold, Dr. Chapman, Dr. Hollingworth, Dr. Gerrish, Dr. Russo, Dr. Hamshere, Dr. Moskvina, N. Jones, C. Thomas, A. Stretton, Prof. Holmans, Prof. O'Donovan, and Prof. Owen contributed to the sample collection, sample preparation, genotyping, and/or conduct of the genome-wide association study upon which this study is based. Dr. Sims and Dr. Harold were responsible for data management and quality control. Dr. Hudson and Dr. Sims carried out the mtDNA SNP analysis under the supervision of Prof. Chinnery. All authors discussed the results and approved the manuscript.

## DISCLOSURE

Dr. Hudson, Dr. Sims, Dr. Harold, Dr. Chapman, Dr. Hollingworth, Dr. Gerrish, Dr. Russo, Dr. Hamshere, Dr. Moskvina, N. Jones, C. Thomas, A. Stretton, Prof. Holmans, and Prof. O'Donovan report no disclosures. Prof. Owen receives/has received research support from GlaxoSmithKline, Medical Research Council UK, and Alzheimer's Research Trust. Prof. Williams is listed as author on a patent re: Identification of variants in loci which are novel risk indicators for the development of Alzheimer's disease and receives/has received research support from GlaxoSmithKline, Medical Research Council UK, the Wales Office of Research and Development for Health and Social Care (WORD), European Union FP6, National Institute for Social Care and Health Research (NISCHR), Alzheimer's Research Trust, Alzheimer's Brain Bank UK (ABBUK), Wellcome Trust, and Fidelity Foundation. Prof. Chinnery is an Honorary Consultant Neurologist at Newcastle upon Tyne Foundation Hospitals NHS Trust; serves as an Associate Editor for *Brain*; is a Wellcome Trust Senior Fellow in Clinical Science and a UK NIHR Senior Investigator; and receives funding from the Medical Research Council (UK), the Parkinson's UK, Association Française contre les Myopathies, and the UK NIHR Biomedical Research Centre for Ageing and Age-related disease award to the Newcastle upon Tyne Foundation Hospitals NHS Trust.

Received July 26, 2011. Accepted in final form November 14, 2011.

## REFERENCES

1. Harold D, Abraham R, Hollingworth P, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet* 2009;41:1088–1093.
2. Hollingworth P, Harold D, Sims R, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet* 2011;43:429–435.
3. Devi L, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK. Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *J Neurosci* 2006;26:9057–9068.
4. Parker WD Jr, Mahr NJ, Filley CM, et al. Reduced platelet cytochrome c oxidase activity in Alzheimer's disease. *Neurology* 1994;44:1086–1090.
5. Lakatos A, Derbeneva O, Younes D, et al. Association between mitochondrial DNA variations and Alzheimer's disease in the ADNI cohort. *Neurobiol Aging* 2010;31:1355–1363.
6. Santoro A, Balbi V, Balducci E, et al. Evidence for sub-haplogroup h5 of mitochondrial DNA as a risk factor for late onset Alzheimer's disease. *PLoS One* 2010;5:e12037.
7. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services

- Task Force on Alzheimer's Disease. *Neurology* 1984;34:939–944.
8. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991;41:479–486.
9. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559–575.
10. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003;19:149–150.
11. Elson JL, Andrews RM, Chinnery PF, Lightowers RN, Turnbull DM, Howell N. Analysis of European mtDNAs for recombination. *Am J Hum Genet* 2001;68:145–153.
12. Torroni A, Achilli A, Macaulay V, Richards M, Bandelt HJ. Harvesting the fruit of the human mtDNA tree. *Trends Genet* 2006;22:339–345.
13. Samuels DC, Carothers AD, Horton R, Chinnery PF. The power to detect disease associations with mitochondrial DNA haplogroups. *Am J Hum Genet* 2006;78:713–720.
14. Ruiz-Pesini E, Mishmar D, Brandon M, Procaccio V, Wallace DC. Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science* 2004;303:223–226.
15. Herrnstadt C, Elson JL, Fahy E, et al. Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences for the major African, Asian, and European haplogroups. *Am J Hum Genet* 2002;70:1152–1171.
16. Hudson G, Carelli V, Spruijt L, et al. Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background. *Am J Hum Genet* 2007;81:228–233.
17. Carelli V, Achilli A, Valentino ML, et al. Haplogroup effects and recombination of mitochondrial DNA: novel clues from the analysis of Leber hereditary optic neuropathy pedigrees. *Am J Hum Genet* 2006;78:564–574.
18. Kruger J, Hinttala R, Majamaa K, Remes AM. Mitochondrial DNA haplogroups in early-onset Alzheimer's disease and frontotemporal lobar degeneration. *Mol Neurodegener* 2010;5:8.
19. Maruszak A, Canter JA, Styczynska M, Zekanowski C, Barcikowska M. Mitochondrial haplogroup H and Alzheimer's disease: is there a connection? *Neurobiol Aging* 2009;30:1749–1755.
20. Mancuso M, Nardini M, Micheli D, et al. Lack of association between mtDNA haplogroups and Alzheimer's disease in Tuscany. *Neurol Sci* 2007;28:142–147.
21. Elson JL, Herrnstadt C, Preston G, et al. Does the mitochondrial genome play a role in the etiology of Alzheimer's disease? *Hum Genet* 2006;119:241–254.
22. van der Walt JM, Dementieva YA, Martin ER, et al. Analysis of European mitochondrial haplogroups with Alzheimer disease risk. *Neurosci Lett* 2004;365:28–32.
23. Carrieri G, Bonafe M, De Luca M, et al. Mitochondrial DNA haplogroups and APOE4 allele are non-independent variables in sporadic Alzheimer's disease. *Hum Genet* 2001;108:194–198.
24. Chagnon P, Gee M, Filion M, Robitaille Y, Belouchi M, Gauvreau D. Phylogenetic analysis of the mitochondrial genome indicates significant differences between patients with Alzheimer disease and controls in a French-Canadian founder population. *Am J Med Genet* 1999;85:20–30.